Plant Mol Biol Rep (2009) 27:563–569
DOI 10.1007/s11105-009-0098-z

# Identification of Cd-Responsive Genes of *Solanum nigrum* Seedlings Through Differential Display

**Jin Xu · Hengxia Yin · Wenying Wang · Qin Mi · Xiaoyong Liao · Xia Li**

Published online: 12 June 2009


**Abstract** *Solanum nigrum* is known as a Cd-hyperaccumulator: Its ability to accumulate large amounts of cadmium in leaves affords its designation as an effective phytoremediator. To identify Cd-responsive genes in *S. nigrum*, a nonradioactive differential display reverse transcription polymerase chain reaction technique was applied to isolate of genes whose transcription was altered in seedlings under Cd stress. A total of 48 DD bands were identified; from these bands, fragments corresponding to seven cDNAs were cloned. Reverse Northern dot-blot analysis confirmed the different expression patterns of these genes under cadmium toxicity. The homology analysis revealed that five of these cDNAs had a clear identity to *Solanum* species, and putative functions were assigned, including calmodulin, ascorbate peroxidase, catalase, glutamylcysteine synthetase, and iron-regulated transporter. Finally, the involvement of these genes in heavy metal tolerance is discussed.

**Keywords** Cadmium toxicity · Nonradioactive differential display · Hyperaccumulator

J. Xu (✉) · X. Li (✉)
Center for Agricultural Resources Research,
Institute of Genetics and Developmental Biology,
Chinese Academy of Sciences,
Huaizhong RD 286,
Shijiazhuang 050021, China
e-mail: xujin@sjziam.ac.cn
e-mail: xli@genetics.ac.cn

H. Yin · W. Wang · Q. Mi
Department of Biology, Qinghai Normal University,
Wusi RD,
Xining, China

X. Liao
Institute of Geographical Sciences and Natural Resources Research,
Chinese Academy of Sciences,
Beijing 100101, China

## Introduction

Heavy metals are components of the ecosystem and can affect the productivity of agricultural crops and the distribution of plant species (Panda 2003). The toxicity of heavy metals, including Cd, Hg, and Pb, strongly inhibits plant growth and development, causing plant death even at very low concentrations. Plants use a variety of mechanisms to assimilate metals while preventing toxicity; they involve the regulation of metal transport across plasma membranes, xylem loading and translocation, detoxification, chelation, and sequestration (Colangelo and Guerinot 2006). Heavy metal stresses inhibit plant growth by disequilibrating the uptake and redistribution of mineral nutrition and disturbing the antioxidant defense system. Cd induces $H_2O_2$ accumulation and reduces the concentrations of all plant nutrients; this has been observed in several plant species (Sanita Di Toppi and Gabbrielli 1999; Sun et al. 2007). The mechanisms used by plants to protect themselves from the potentially massive influx of heavy metal ions into the cytoplasm under heavy metal stresses are not fully understood (Arrivault et al. 2006).

The traditional differential display reverse transcriptase-polymerase chain reaction (DDRT-PCR) method (Liang and Pardee 1992) has been used successfully in many cases. This method has the advantage of technical simplicity and lower bias against rare messages (Iqbal et al. 2008). However, a number of bands from polyacrylamide gel are too low for effective cloning of desired products. Several modifications of the original approach have been reported to minimize some of these problems (Kim et al. 2004; Iqbal et al. 2008). The products of DDRT-PCR are resolved and observed on agarose gels by the modified technique; therefore, the number of low molecular weight amplification products is reduced, and the differentially expressed genes are easily identified.

*Solanum nigrum* is known as a Cd-hyperaccumulator occurring in soils contaminated with cadmium. Its ability to uptake and accumulate large amounts of cadmium in its leaves make this plant an effective phytoremediator (Wei et al. 2006). In recent years, the physiological characterization of *S. nigrum* under cadmium stress has been reported (Sun et al. 2007). To our knowledge, cloning the differentially expressed genes in response to Cd exposure in *S. nigrum* has not been carried out. In the present study, we employed the nonradioactive mRNA differential display technique to identify genes differentially expressed under Cd stress. In total, 48 DD bands were identified; from these bands, fragments corresponding to seven cDNAs were cloned. Reverse Northern dot-blot analysis was performed to confirm and analyze the different expression patterns of these genes under cadmium toxicity. Finally, the involvement of these genes in heavy metal tolerance is discussed.

## Materials and Methods

### Plant Materials, Growth Conditions, and Stress Treatments

Seeds of *S. nigrum* were kindly gifted by the National Southwest Germplasm Resources Lab, China (IP: SCSB-C-003073) in October 2007 and then stored at −20°C until use. Seeds were sown under sterile conditions in Petri dishes containing Murashige and Skoog medium, solidified with 0.8% (*w*/*v*) agar (Sigma). Cultures were maintained at 25°C under a 16-h photoperiod with a photosynthetic photon flux density of 45 μmol $m^{-2}$ $s^{-1}$. After germination, the seedlings were transferred into Hoagland solution for plant growth under the same culture conditions. Seven-day-old seedlings were treated with different concentration of $CdSO_4$ for different time periods.

### Determination of Growth and Cd Accumulation Under Cadmium Stress

After Cd stress treatments, the root length was determined. For Cd accumulation analysis, the seedlings were rinsed with deionized water and then oven-dried. Dried plant tissues were ground up and digested in concentrated nitric acid for 2–3 days at room temperature. Samples were then boiled until they were completely digested, diluted with Millipore-filtered deionized water, and briefly centrifuged. The concentrations of Cd were determined by atomic absorption spectrometry (SHIMADZU AA-6300).

### Differential Display Reverse Transcription-Polymerase Chain Reaction

After exposure to 100 μM $CdCl_2$ for 72 h, total RNA was isolated from *S. nigrum* seedlings using TRIZOL reagent (Gibco BRL, USA). The cDNAs were synthesized from DNase-treated total RNA with a Reverse Transcription System kit (Promega, USA). Three sets of 3′-oligo(dT) anchored primers (AP1–3) were used in combination with one of the seven 5′-arbitrary primers (RP1–7) for PCR amplification (Table 1). PCR was conducted in 0.2-mL thin-walled tubes in a MJ Research Minicycler. The reaction mixture, in a total volume of 50 μl, contained 1× reaction buffer, 10 mM dNTP mixture, 2 μl anchored primer (250 ng/μl), 8 μl of arbitrary primer (100 ng/μl), 1 μl Taq polymerase, and 10 ng of the template DNA. The reaction volume was brought up to 50 μl using sterile $dH_2O$. The PCR cycling conditions included an initial denaturation step at 94°C for 4 min. This was followed by 35 cycles with denaturation at 94°C for 30 s, annealing at 36°C for 30 s, and elongation at 72°C for 45 s. A final extension step at 72°C for 10 min was included. PCR products were electrophoresed on a 1% agarose gel, stained in ethidium bromide, and the DNA visualized under ultraviolet light. In order to produce differential display banding patterns, all steps were carried out in duplicate to prevent false positives. Compared with those of control plants, the cDNAs differentially expressed in the seedlings of stressed plants were excised from the gel and extracted with a gel extraction kit (QIAGEN). The eluted DNA samples were used as the template for reamplification using the appropriate primer pairs. Successfully reamplified cDNA fragments were gel-purified and cloned into the pGEM-T Easy vector (Promega, USA), followed by sequencing. The nucleotide sequences were screened for identification with Basic Local Alignment Search Tool (BLAST) software from National Center for Biotechnology Information (NCBI).

**Table 1** Sequence of the 5′-RP (arbitrary primers) and 3′-oligo(dT) primers (anchored primers) that were used in differential display-PCR

| | Primer sequences |
|---|---|
| Anchored primers | |
| AP1 | AAGCTTTTTTTTTTA |
| AP2 | AAGCTTTTTTTTTC |
| AP3 | AAGCTTTTTTTTTG |
| Arbitrary primers | |
| RP1 | CAATCGCCGT |
| RP2 | GTTCCGCCCC |
| RP3 | AAGTACTTAG |
| RP4 | AAGAGCCCGT |
| RP5 | CCCCGGTAAC |
| RP6 | TCCCGCTGCG |
| RP7 | GTCCGTGAGT |

### Reverse Northern Dot-Blot Analysis

For the reverse Northern dot-blot analysis, previously cloned differentially expressed cDNAs were amplified using appropriate primers. The cDNAs were amplified and purified. Purified cDNA (300 ng) was denatured with 0.1 M NaOH (final concentration) and incubated at 100°C for 5 min. The solution was neutralized with the addition of 3× saline–sodium citrate (final concentration), and then the volume was adjusted to 75 μl with distilled $H_2O$. Then, 8 μl of each sample were manually applied to one of nine nylon membranes and baked for 30 min at 80°C (Lang et al. 2005). Single-stranded digoxigenin (DIG)-labeled cDNA probes were prepared from total RNA isolated from control and metal stress-treated *S. nigrum* seedlings according to manufacturer's instructions. Equal amounts of each DIG-labeled cDNA mixture were denatured and separately hybridized to one of the nine membranes according to manufacturer's instructions (Roche, DIG High Prime DNA Labeling and Detection Starter Kit II). The expression level of each cDNA for a differentially expressed gene was obtained from the ratio between the stressed value and the control value after normalization to the expression of 16S RNA.

## Results

### Plant Growth and Cd Accumulation

To select an appropriate concentration and time course for Cd exposure, we first investigated the effects of different concentrations of Cd on the growth of *S. nigrum* seedlings and metal accumulation. As shown in Fig. 1, the effects of Cd on root growth varied with the different Cd concentrations used. We determined that 50 μM of Cd had a slight stimulatory effect on root elongation during the first 24 h. However, the root elongation decreased with an increased Cd concentration. Seedlings exposed to 250 μM of Cd exhibited substantial growth reduction, and root growth eventually stopped. After 24 h of exposure to 100 μM Cd, root elongation decreased to 50.7% of that in untreated control plants; hence, 100 μM was chosen as a sublethal dosage in the experiment. The accumulation of $Cd^{2+}$ in the seedlings increased with increasing Cd concentrations and duration of treatment (Fig. 2). The highest Cd accumulation in *S. nigrum* seedlings occurred between 72 and 96 h after the onset of Cd exposure; hence, a greater number of genes for Cd accumulation and tolerance should be fully expressed at 72 h. Therefore, we selected 100 μM of Cd concentration and a 72-h time course of Cd exposure to evaluate the Cd-responsive genes in *S. nigrum*.

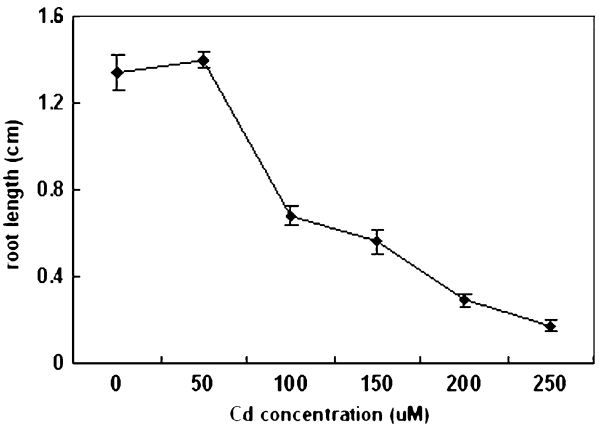


**Fig. 1** Root elongation of *S. nigrum* seedlings after 24 h of exposure to different concentrations of Cd (0–250 μM). Each value is the mean of six replicates and *vertical bars* represent ± standard error

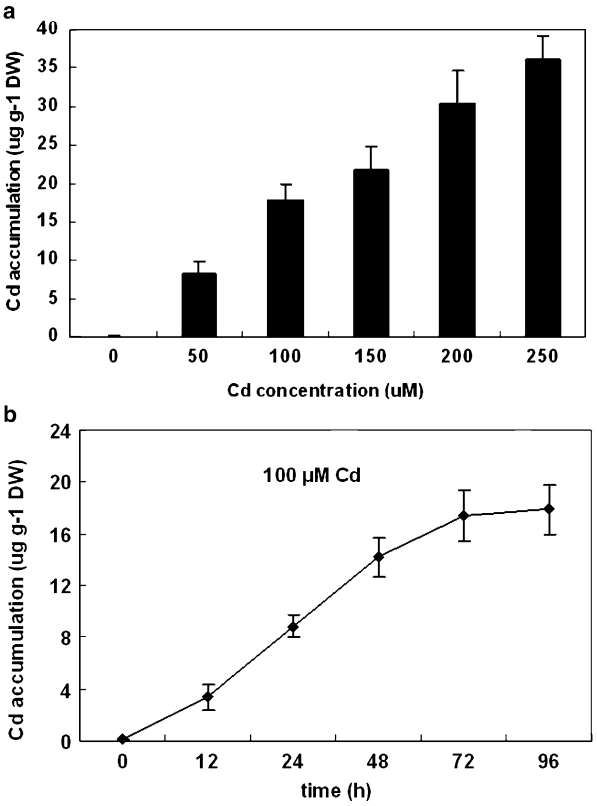


**Fig. 2** Accumulation of Cd in the seedlings of *S. nigrum*. Each value is the mean of six replicates and *vertical bars* represent ± standard error

### Identification of Cd-Responsive Genes by the Nonradioactive mRNA Differential Display Technique and Further Confirmation by Reverse Northern Dot Blot

In order to identify new differentially expressed genes, cDNA samples were obtained from control and stressed *S. nigrum* seedlings. Three sets of 3′-oligo(dT) anchored primers (AP1–3) were used in combination with one of

**Table 2** Homologies of sequences of differential display-PCR clones to sequences in databases

| Clone | Primer pair used in PCR | Size of PCR (bp) | Sequences homology | Best *E* value | GenBank accession no. |
|---|---|---|---|---|---|
| DD1 | RP7/AP3 | 169 | *S. tuberosum* clone 121A05 ascorbate peroxidase-like mRNA | 2e−82 | GE650053 |
| DD2 | RP7/AP3 | 246 | calmodulin 5/6/7/8-like protein | 2e−11 | GE650054 |
| DD3 | RP7/AP3 | 191 | *L. esculentum* catalase mRNA | 0 | GE650047 |
| DD4 | RP4/AP2 | 514 | *S. lycopersicum* DNA sequence from clone LE_HBa-291F9 on chromosome 4 | 0.005 | GE745609 |
| DD5 | RP7/AP3 | 167 | *L. esculentum* glutamine synthetase | 1e−29 | GE650049 |
| DD6 | RP1/AP1 | 197 | *C. annuum* clone B0320-195 mRNA sequence | 2e−31 | GE745610 |
| DD7 | RP3/AP2 | 207 | *L. esculentum* iron-regulated transporter | 2e−37 | GE650052 |

the seven 5′-arbitrary primers (RP1–7) for PCR amplification. In total, 48 bands were identified; from these bands, fragments corresponding to seven cDNAs were cloned (Table 2). Six of these cDNA bands showed an increase in band intensity in Cd-treated samples, whereas the DD6 cDNA band showed decreased band intensity in the Cd-treated samples. Representative differential display data are shown in Fig. 3. The size of the cloned cDNA fragments ranged from 167 to 514 bp (Table 2). These cDNAs were successively confirmed by reverse Northern dot blot (Table 3; Fig. 4). In Cd-treated samples, the expression levels of DD6 showed a 2.31-fold decrease, whereas 1.71-, 1.86-, 4.22-, 4.94-, 1.51-, and 2.18-fold increases were observed for DD1, DD2, DD3, DD4, DD5, and DD7, respectively (Table 3). DD4 showed the highest level of expression during stress among the seven cDNAs of differentially expressed genes.

## Sequencing and Identification of cDNA Clones

The nucleotide sequences of the Cd-responsive cDNAs were determined and compared with those in GenBank Nucleotide Sequence Databases through the NCBI blast server to identify putative proteins encoded by these mRNAs. The results of this analysis were summarized in Table 2. The DD1, DD2, DD3, DD5, and DD7 clones showed significant homology to ascorbate peroxidase of *Solanum tuberosum* (*E*=2e−82), calmodulin of *S. tuberosum* (*E*=2e−11), catalase (CAT) of *Lycopersicon esculentum* (*E*=0), glutamine synthetase of *L. esculentum* (*E*=1e−29), and iron-regulated transporter (IRT) of *L. esculentum* (*E*=2e−37), respectively. Clones DD4 and DD6 showed homologies to the *Solanum lycopersicum* DNA sequence from clone LE_HBa-291F9 (*E*=0.005) and to the *Capsicum annuum* clone B0320-195 mRNA sequence

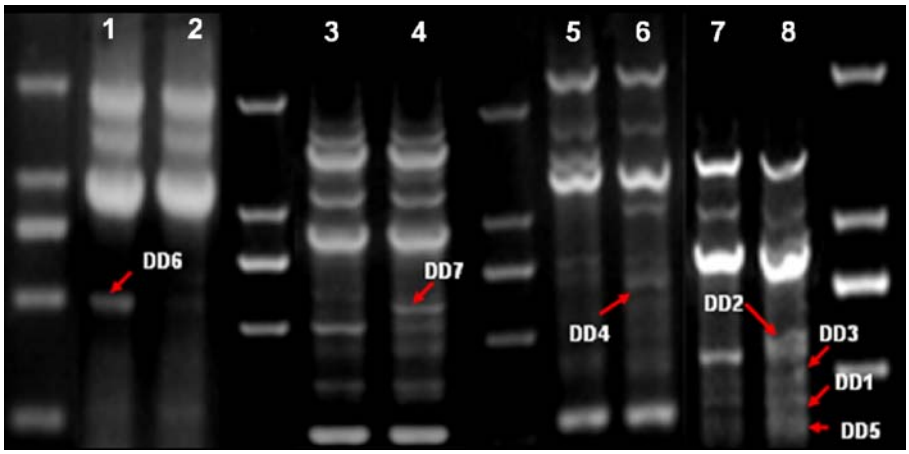


**Fig. 3** Differential display band patterns of mRNA from seedlings of control and Cd-treated *S. nigrum*. Total RNAs from control (*ck*) and 72-h Cd-treated *S. nigrum* were reverse-transcribed and amplified with the 5′-arbitrary primer and the 3′-oligo(dT) primer. Amplified cDNA fragments were electrophoresed on a 1% agarose gel and stained in ethidium bromide, then the DNA was visualized under ultraviolet light. Control: *lanes 1*, *3*, *5*, *7*; Cd treated: *lanes 2*, *4*, *6*, *8*; primer pairs used in *lanes 1*, *2*: RP1/AP1; *lane 3*, *4*: RP3/AP2; *lanes 5*, *6*: RP4/AP2 and *lanes 7*, *8*: RP7/AP3

**Table 3** Results of the reverse Northern dot-blot analysis

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| A | | | | |
| a | 16S RNA | DD1 | DD2 | DD3 |
| b | DD4 | DD5 | DD6 | DD7 |
| B | | | | |
| a | 1.00 | 1.71 | 1.86 | 4.22 |
| b | 4.94 | 1.51 | −2.31 | 2.18 |

*A* positions of seven cDNAs corresponding to differentially expressed genes and 16S RNA cDNA on the dot-blot membranes, *B* expression levels of the corresponding cDNAs of differentially expressed genes obtained from the ratio between stressed value and control value after normalization to the 16S RNA expression

($E$=2e−31), respectively, suggesting that they may either encode unidentified proteins or correspond to untranslated regions of mRNA.

## Discussion

*S. nigrum* is an effective phytoremediator (Wei et al. 2006). In recent years, the physiological characterization of *S. nigrum* under heavy metal stress has been reported (Wei et al. 2006; Sun et al. 2007, 2008). However, identification of Cd-responsive genes in *S. nigrum* has not been carried out. Research on the Cd-responsive genes would be helpful to further elucidate the mechanism of Cd tolerance and accumulation in *S. nigrum*. Using the nonradioactive DDRT-PCR technique, we have identified a number of genes whose expression was altered under Cd stress. In total, seven of the bands obtained from 84 PCR reactions appeared to be differentially expressed; they were successively confirmed by reverse Northern dot-blot analysis and identified by comparison with sequences available in the GenBank Nucleotide Sequence Databases. These gene products are involved in metabolism, intracellular transport, and stress responses.

The sequence of DD2 showed a high identity with calmodulin of *S. tuberosum*, *NtCaM12* of *Nicotiana tabacum*, and calmodulin of *Oryza sativa*. Calmodulin, the predominant calcium receptor, is one of the best-characterized calcium sensors in eukaryotes; it regulates diverse cellular functions by modulating the activity of a variety of enzymes and proteins. Previous studies suggest that $Ca^{2+}$ and calmodulin are involved in various stress responses. The increase of the *S. nigrum* calmodulin transcript level suggests that Cd may also activate the calmodulin signaling pathway.

The DD1 and DD3 clones encode ascorbate peroxidase and catalase, which involved in the defenses against oxidative stress induced by biotic and abiotic stresses in plants (Singh and Tewari 2003). Cd toxicity induces significant intracellular $H_2O_2$ accumulation (Dixit et al. 2001). Wang et al. (2008) found that the activities of superoxide dismutase (SOD) and CAT in the metal accumulators *Brassica juncea* and *Thlaspi caerulescens* were significantly higher than that in nonaccumulator tobacco under normal conditions. These authors also showed that the activities of SOD and CAT increased rapidly in metal accumulators under Cd stress, indicating that antioxidative enzymes in hyperaccumulators are important components of the antioxidant defense mechanisms to combat Cd-induced oxidative stress. *S. nigrum* accumulated high concentration of cadmium in shoots; hence, the increases of *S. nigrum* ascorbate peroxidase and catalase transcripts could maintain a favorable cellular $H_2O_2$ concentration to protect the cell from being destroyed by oxidative stress and to improve the cadmium accumulation in shoots of *S. nigrum*.

Using DDRT-PCR, we have isolated and identified a gene encoding glutamine synthetase (clone DD5) in *S. nigrum*. The reverse Northern dot-blot analysis showed an increase of this transcript after 3 days of Cd treatment. Glutamine synthetase plays a role in the flow of nitrogen into nitrogenous organic compounds by catalyzing the assimilation of ammonia into glutamine, which is then converted to glutamate via the action of glutamate synthase (Li et al. 1993; Rana et al. 2008). Glutamine regulates the cellular redox balance, including oxidative metabolism, apoptosis, and cell proliferation in mammalian cells (Matés et al. 2002). Glutamine also is a substrate for protein synthesis, an interorgan nitrogen transporter and a precursor for glutathione production. Many of these functions are connected to the formation of glutamate from glutamine (Matés et al. 2002). Therefore, the glutamine/glutamate metabolism plays a central role in heavy metal tolerance

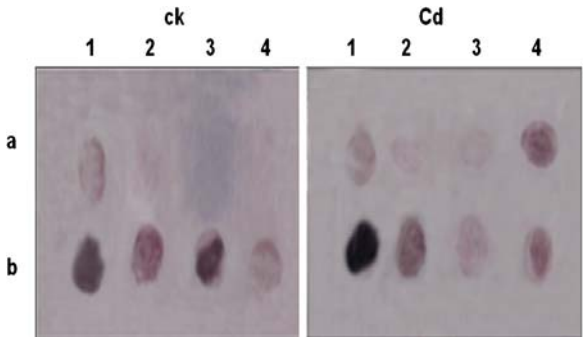


**Fig. 4** Reverse Northern dot-blot analysis of differentially expressed genes. Cloned cDNAs were amplified, denatured, and blotted on two nylon membranes as described in Table 3. Membranes were hybridized with DIG-labeled total cDNAs from control (*left*) and cadmium stress (*right*) plants

and accumulation. Previous studies have revealed that Cd detoxification depends, to a significant degree, on glutathione and phytochelatin production in *S. nigrum* (Sun et al. 2007). A possible role of glutathione is to reduce the concentration of free metal ions in the cell and prevent an increase in the production of reactive oxygen species under heavy metal stress (Xu et al. 2009). Massive Cd accumulation and transport from root to shoot require increased glutathione and phytochelatin synthesis. Phytochelatin is enzymatically synthesized from glutathione. High glutathione levels facilitate phytochelatin synthesis and sequestration of heavy metal phytochelatin conjugates in the vacuole, thereby enhancing the cadmium accumulation and transport from roots to shoots in plants (Siripornadulsil et al. 2002; Mendoza-Cozatl and Moreno-Sanchez 2006). Glutamine/glutamate metabolism mediates the glutathione biosynthesis (Li et al. 1993; Matés et al. 2002). Therefore, the elevated expression of the glutamine synthetase gene in Cd-induced *S. nigrum* seedlings indicated that accumulation of glutamine is an important mechanism of Cd tolerance and accumulation in the hyperaccumulator *S. nigrum*.

DD7 shows high identity with *L. esculentum* IRT mRNA. IRTs are members of the zinc and iron-regulated transporter protein (ZIP) metal transporter family that are associated with zinc and iron uptake. There are 17 members of the ZIP transporter gene family that have been identified in the *Arabidopsis* genome. *Arabidopsis* IRT1 is thought to function as a broad specificity iron transporter that facilitates the transport and accumulation of zinc and cadmium in plants during iron deficiency (Cohen et al. 1998; Connolly et al. 2002). *TcZNT1* is a member of the ZIP family in the hyperaccumulator *T. caerulescens*. Overexpression of *TcZNT1* increased zinc influx in roots and accumulation in shoots indicating that *ZNT1* is responsible for zinc absorption and transport from root to shoot in *T. caerulescens*. The increase of *S. nigrum* IRT mRNA transcripts implies that it plays a role in Cd accumulation and/or rescuing the iron deficiency in *S. nigrum* induced by Cd exposure. Cd tolerance and accumulation is a network of multiple mechanisms that are finely organized in plants. Wei et al. (2005) reported that the Cd enrichment factor (ratio of Cd concentrations in plant and soil) in shoots of *S. nigrum* was as high as 2.68, and the Cd accumulation in shoots was greater than that in roots, indicating a higher cadmium absorption and root-to-shoot transport capacities in the hyperaccumulator *S. nigrum*. Future experiments will determine the different expression patterns of these differentially expressed genes in shoots and roots of *S. nigrum* seedlings and whether Cd hyperaccumulation in *S. nigrum* contributes to the enhancement of the root-to-shoot transport.

Most of the Cd-hyperaccumulators belong to the *Brassica* family; however, *Sedum alfredii* (Lu et al. 2008) and *S. nigrum* are nonbrassica Cd hyperaccumulators. Researches on the physiological, biochemical, and molecular mechanisms of Cd hyperaccumulation are necessary to further optimize the potential use of Cd hyperaccumulators for phytoremediation (Salt et al. 1998; Lu et al. 2008). In the present study, we identified seven cDNAs including five reported Cd upregulated genes in plants and two new transcripts that responded to Cd toxicity. A higher number of random decamers might be utilized to detect a greater number of differentially expressed genes from Cd-treated *S. nigrum*. The regulation of gene expression to cope with Cd exposure in hyperaccumulators differs from that in sensitive species. Further functional studies of these Cd-responsive genes will clarify their role in heavy metal tolerance and hyperaccumulation and are essential for the application to phytoremediation.

**Acknowledgments** We thank Dr. Dezhu Li of Kunming Institute of Botany, Chinese Academy of Sciences for providing the *S. nigrum* seeds. The authors truly appreciate the time that Dr. Schuyler Korban and the anonymous reviewers spent on helping to clarify the confusions and modify the paper. The research was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (grant no. 0707013603).

## References

Arrivault S, Senger T, Kramer U (2006) The *Arabidopsis* metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply. Plant J 46:861–879

Cohen CK, Fox TC, Garvin DF, Kochian LV (1998) The role of iron-deficiency stress responses in stimulating heavy-metal transport in plants. Plant Physiol 116:1063–1072

Colangelo EP, Guerinot ML (2006) Put the metal to the petal: metal uptake and transport throughout plants. Curr Opin Plant Biol 9:322–330

Connolly EL, Fett JP, Guerinot ML (2002) Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. Plant Cell 14:1347–1357

Dixit V, Pandey V, Shyam R (2001) Differential oxidative responses to Cd in roots and leaves of pea (*Pisum sativum* L cv. Azad). J Exp Bot 52:1101–1109

Iqbal S, Bashir A, Naseer HM, Ahmed M, Malik KA (2008) Identification of differentially expressed genes in developing cotton fibers (*Gossypium hirsutum* L.) through differential display. Electron J Biotechn 11:1–10

Kim YJ, Kwak C, YY GU, Hwang IT, Chun JY (2004) Annealing control primer system for identification of differentially expressed genes on agarose gels. Biotechniques 36:424–434

Lang ML, Zhang YX, Chai TY (2005) Identification of genes up-regulated in response to Cd exposure in *Brassica juncea* L. Gene 363:151–158

Li MG, Villemur R, Hussey PJ, Silflow CD, Gantt JS, Snustad DP (1993) Differential expression of six glutamine synthetase genes in Zea mays. Plant Mol Biol 23:401–407

Liang P, Pardee AB (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257:967–971

Lu LL, Tian SK, Yang XE, Wang XC, Brown P, Li TQ, He ZL (2008) Enhanced root-to-shoot translocation of cadmium in the hyperaccumulating ecotype of *Sedum alfredii*. J Exp Bot 59:3203–3213

Matés JM, Pérez-Gómez C, Núñez de Castro I, Asenjo M, Márquez J (2002) Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death. Int J Biochem Cell Biol 34:439–458

Mendoza-Cozatl DG, Moreno-Sanchez R (2006) Control of glutathione and phytochelatin synthesis under cadmium stress. J Theor Biol 238:919–936

Panda SK (2003) Heavy-metal phytotoxicity induces oxidative stress in a moss, *Taxithellium* sp. Curr Sci 84:631–633

Rana NK, Mohanpuria P, Yadav SK (2008) Expression of tea cytosolic glutamine synthetase is tissue specific and induced by cadmium and salt stress. Biol Plant 52:361–364

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668

Sanita Di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Singh PK, Tewari RK (2003) Cd toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. J Environ Biol 24:107–112

Siripornadulsil S, Traina S, Verma DPS, Sayre RT (2002) Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. Plant Cell 14:2837–2847

Sun RL, Zhou QX, Sun FH, Jin CX (2007) Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, *Solanum nigrum* L. Environ Exp Bot 60:468–476

Sun YB, Zhou QX, Diao CY (2008) Effects of cadmium and arsenic on growth and metal accumulation of Cd-hyperaccumulator *Solanum nigrum* L. Bioresource Technol 99:1103–1110

Wang Z, Zhang YX, Huang ZB, Huang L (2008) Antioxidative response of metal-accumulator and non-accumulator plants under cadmium stress. Plant Soil 310:137–149

Wei SH, Zhou QX, Wang X, Zhang KS, Guo GL, Ma Lena QY (2005) A newly-discovered Cd-hyperaccumulator *Solanum nigrum* L. Chin Sci Bull 50:33–38

Wei SH, Zhou QX, Koval PV (2006) Flowering stage characteristics of Cd hyperaccumulator *Solanum nigrum* L. and their significance to phytoremediation. Sci Total Environ 369:441–446

Xu J, Yin HX, Li X (2009) Protective effects of proline against cadmium toxicity in micropropagated hyperaccumulator, *Solanum nigrum L*. Plant Cell Rep . doi:10.1007/s00299-008-0643-5